REGENERATION AND REGULATION IN PARAMECIUM CAUDATUM.

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The investigations described here were begun in February, 1910, upon some pure lines supplied through the courtesy of Professor Jennings. The experiments were undertaken with the hope of determining how new races arise, and if possible, to produce by artificial means, a new race differing in size and perhaps in other characteristics from the parent stock.

A long series of experiments was carried on, testing the effect of various foods, and also subjecting the organisms to other changes in the environment. The conjugation of a large and a small individual was followed in order to see if the size of the ex-conjugants was modified by the inequality of the gametes. It was found impossible by any of these methods to produce a race with new characteristics.

In the winter of 1910–1911 a study of the regenerative and regulative power of single individuals and of conjugating pairs was undertaken. It was hoped that after the removal of part of the cytoplasm, and possibly some of the nuclear material as well, a small race might be produced. It soon became evident that the removal of the nuclear material was not possible, for no cell or fragment of a cell (unless cut while in the process of division) recovered after the nucleus was injured. Owing to the extremely small number of successful operations, and the alluring interest of various side issues which arose in connection with the work the main problem was neglected for a time and other experiments, which will be described in this paper, have been carried on at intervals up to the present date.

In November, 1910, in a paper read before the Cambridge Philosophical Society, Levin ('10) described very briefly some experiments on *Paramecium* in which he produced races without a micronucleus, and others where he found it possible to divide a living cell so that each fragment received a portion of the meganucleus. The brevity of the paper makes it difficult to determine how this result was brought about. I have not been able to find a more complete account of these preliminary experiments.

In June, 1911, while working in Professor Boveri's laboratory in Würzburg, I made a series of experiments on large races of *Paramecia* from Munich and Würzburg, and up to May, 1912, these pure lines were used for comparison with material collected in Bryn Mawr. During the course of my investigations a paper appeared by Calkins ('11) in which he describes in detail the behavior of fragments of *Paramecia* after the removal of some of the cytoplasm. My results confirm those of Calkins so exactly that I shall omit a full description of the individual experiments merely giving a summary of the results in tabulated form.

It gives me pleasure to have this opportunity to express my gratitude to Professor Boveri for the many courtesies extended to me during my stay in Würzburg, and to Professor Jennings for his kindness in supplying me with some of his pure lines.

Methods.

The methods followed were practically those used by Calkins except that instead of treating the cells with neutral red before cutting them, the animals were quieted by placing them in a small drop of tap water thickened with quince seed. Immediately after the operation a few drops of culture fluid were added. All cultures were kept in hollow slides in a moist chamber. The culture medium was changed every twenty-four to forty-eight hours as conditions required. The infusion made from Timothy heads, according to Jennings' method, was the culture fluid most generally employed. A most satisfactory culture medium if rightly used is a .2 per cent. solution of Horlick's Malted Milk.

Experiments.

A. *The Behavior of Cells after Removal of Part of the Cytoplasm.*

Thousands of *Paramecia* were cut during the course of these experiments. The fragments were examined four hours after the operation and then again on the following day. All fragments living less than twenty-four hours after the operation were omitted from the records. By this process of elimination the making of tables was greatly simplified.

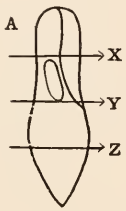


FIG. 1. Diagram of vegetative cell showing regions of the cut for removing the anterior or posterior end and dividing the cell in half.

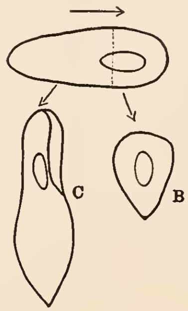


FIG. 2. Fragment of cell from which the anterior end was removed. B and C, the cells formed from it by division in the original plane.

1. *Removal of the Anterior End.*—In this experiment the anterior end of the cell was cut off at the level indicated in Fig. 1, *X*, thus removing from one fourth to one third of the cytoplasm. The small non-nucleated piece (Fig. 1, *A*) anterior to the cut usually disintegrates immediately after the operation, but in a few cases it closed in, forming a spherical mass which swam about rapidly for a day and then died. Such pieces never divide or show any sign of growth. The removal of the end often produces great disturbance in the larger nucleated piece. This is probably due to the fact that the macronucleus lies so near the level of the cut, and the oral groove prevents the closure of the wound. The following table gives the results from eighty-three individuals.

TABLE I.

Number of Paramecia.	Regeneration Followed by Normal Fission.	Division in Original Plane.	No Division.	Monsters.	Killed.
83	28	42	8	4	2

Thirty-four per cent. of the pieces developed new cilia at the cut end and grew to the normal size before dividing, but nearly fifty per cent. divided in the original plane (represented in all figures by the dotted line) forming a small irregular anterior cell (Fig. 2, *B*) and a normal posterior cell (*C*). The smaller cell may grow rapidly to the normal size and divide into two equal cells, or it may form another small anterior and large posterior cell. At times the anterior cell finally produces a monster.

2. *Removal of the Posterior End.*—The removal of the posterior end of the cell through the region back of the mouth (Fig. 1, *Z*) produces less disturbance than cutting off the anterior end. This result would be expected as there is little danger of injury to the nucleus which lies in front of the cut. The results from this experiment are given in the next table.

Table II.

Number of Paramecia.	Regeneration Followed by Normal Fission.	Division in Original Plane.	No Division.	Monsters.	No Regeneration.
88	55	18	14	1	1

The percentage of fragments which regenerated and divided in the normal manner is much larger than that of Table I. Here sixty-two per cent. after recovery from the operation divided normally, forming a race of the original size, while only thirty-four per cent. of the cells in Table I. regenerated the lost end before dividing. In twenty per cent. of the fragments the division was irregular, resulting in a large anterior and a small posterior cell. This result stands out in sharp contrast to the fifty per cent. dividing irregularly in Table I. The probable explanation of this result is to be found in the fact that when the anterior end is removed the nucleus is either exposed directly to the surrounding medium, or is separated from it by a thin film of cytoplasm, and may be thus stimulated to divide in the plane already laid down before a re-arrangement of symmetry is effected. The result is that one daughter cell contains twice as much cytoplasm as the other, while both have the same amount of nuclear material. When the posterior end is removed (Fig. 1, *Z*) there is an equal amount of cytoplasm back and front of the nucleus, so that a new division plane forms, and when fission takes place each half gets an equal amount of cytoplasm. The later history of the small cells formed by irregular division will, I think, support this suggestion.

B. *The Behavior of Cells Cut in Half Transversely.*

It is extremely difficult to cut a *Paramecium* in half without permanently injuring both halves. The macronucleus almost invariably slips out and the pieces disintegrate within an hour or two. If, however, the nucleus is pushed forward or backward, as the case may be, into one of the halves that piece lives and will finally divide into two normal individuals. Out of forty cells thirty died without fission, and ten produced new races of the normal size.

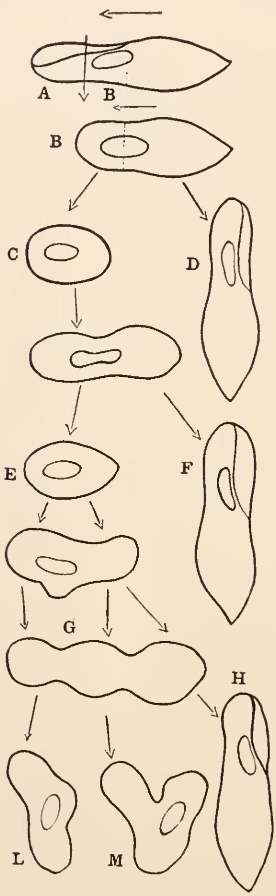


Fig. 3. Diagram giving the history of the formation of three normal races and two monsters from a cell from which the anterior end was cut off.

All of the experiments described so far were made, without exception, upon cells in the vegetative condition. None of the cells had recently divided or were preparing to divide.

C. *The Formation of Monsters.*

Calkins ('11) has shown that it is possible to produce from a small fragment a mass of such dimensions and structure that it represents many individuals, having several mouths, oral grooves, and vacuoles. I have not succeeded in obtaining more than a dozen of these monsters in the course of my investigations. Most of them developed during the warm weather in the latter part of June. Thinking that the temperature had something to do with their appearance I began a series of experiments under various degrees of warmth above that of the laboratory, but did not succeed in producing any more monsters than at room temperature. A brief description of several of these monsters may throw some light on the question of their mode of origin.

No. 1.—This monster arose from one of the fragments in Table I. where the anterior end of the cell was removed (Fig. 3). At the end of twenty-four hours the fragment (*B*) appeared active but showed no sign of fission; the cut end was slightly rounded. Before the end of forty-eight hours division took place in the original plane producing a small individual (*C*) and a large one (*D*). On the following day *C* began to divide. During the next night fission was completed and another small (*E*) and large (*F*) cell formed. On the fourth day *E* had increased in size, and showed signs of division. On the fifth day it had formed a chain of three cells (*G*), the most posterior of which formed a normal individual. The chain continued to throw off

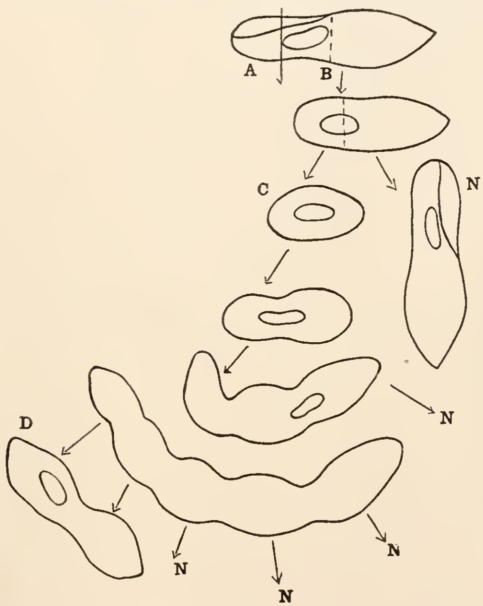


FIG. 4. Diagram showing the formation of five normal races and one monster.

normal cells posteriorly until finally after a period of growth a mass was left which separated into two parts (*L* and *M*). These when killed and stained were found to possess several mouths, but neither piece contained more than one nucleus.

No. 2.—The second monster arose in the same way from a small anterior cell produced by the division of a fragment in the original plane. In this case a chain of five parts (Fig. 4) formed from the small piece (C). Finally the chain divided posteriorly into a series of normal individuals (N) leaving anteriorly one double cell (D). On the seventh day this monster was killed and stained. Three mouths, two vacuoles, and only one nucleus were present.

No. 3.—The third monster (Fig. 5) formed directly from the small anterior piece (C) without dividing. The piece grew rapidly for three days developing processes resembling budding individuals but none were thrown off. At the end of the third day the mass (m) was killed. Again only one nucleus was found but three mouths and four vacuoles were present.

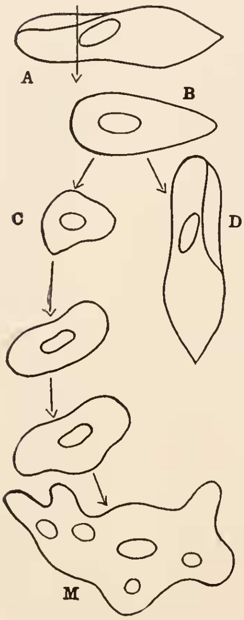


Fig. 5. Diagram showing the formation of a monster from a small cell without division.

Such cases as these demonstrate that there is great disturbance produced through the removal of some of the cytoplasm of a vegetative cell. The small piece (Fig. 5, C) contained all of the nuclear material for a normal cell, but less than half of the protoplasm required. Before the cell-body has attained its full growth the nucleus is ready to divide, and in turn when the cytoplasm is ready to divide the nucleus is not. This condition of affairs produces a complete loss of balance in the mechanism of division and the result is that irregular cells and monsters are formed and the cell rarely ever regains its normal condition.

D. *The Effect upon Cells Cut During Division.*

For these experiments *Paramecia* were selected which were in the early stages of fission, at a time when the nucleus was elongated and the body slightly constricted (Fig. 6).

Experiment 1.—When the cell is cut in half through the plane of division (Fig. 6, Y) both cytoplasm and nucleus are divided equally. The experiment is a comparatively simple one and is usually successful, both halves living and forming normal races.

The survival of both pieces depends largely on the stage of fission reached at the time of the operation. The nearer the process is to completion the more likely is the recovery of both halves.

Experiment 2.—When the cut was made through the center of the anterior or the posterior half of the dividing cell (Fig. 6, *X* and *Z*) the small non-nucleated piece always died, and the large piece continued to divide in the original plane forming a small cell (Fig. 7, *C*) and a large cell (*D*).

Experiment 3.—When the cut is made in either half of the dividing cell near the plane of division (Fig. 8, *X* and *Y*) the

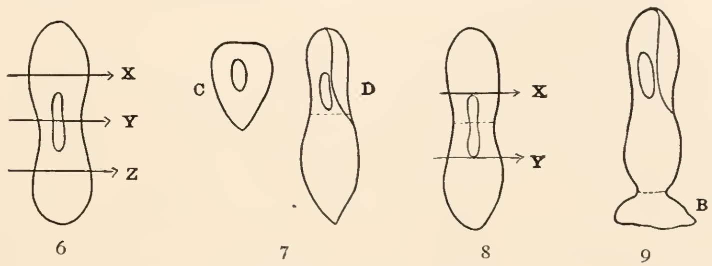


FIG. 6. Dividing cell showing the region of the cuts.

FIG. 7. Small and large individual formed by division in the original plane after removal of the anterior end of a cell during the process of fission.

FIG. 8. Diagram showing cuts made near the center of a dividing cell.

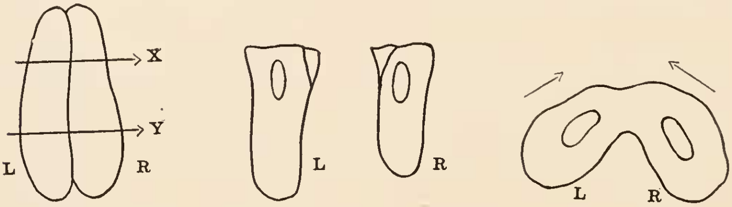
FIG. 9. Cell with fragment of the posterior half still attached.

small fragment (Fig. 9, *B*) remains attached to the other half, and is gradually absorbed. In a few cases division continued in the original plane, but this occurred only in those cells where fission was almost completed at the time of the operation.

E. *The Effect of Cutting Cells During Conjugation.*

During epidemics of conjugation pairs which had become firmly united were isolated and from these either the anterior or the posterior ends were removed (Fig. 10, *X* and *Y*). None of the pairs were cut through the center. The non-nucleated fragments always died shortly after the operation, while the larger ones either remained in contact for several hours or separated at once. Regeneration in these pieces is exceedingly slow and fission is greatly delayed. Truncated fragments (Fig. 11.

L and *R*) have been observed for days after the operation, swimming about rapidly but showing no sign of fission, or of regeneration of the lost end. Although the conjugating pairs were cut at the same level after separation one fragment was almost always smaller than the other (Fig. 11). The small one (*R*) generally dies, and the large one (*L*) divides to form a normal race, when both halves lived the smaller one finally divided and after many generations the descendants regained the normal size. The fission of all fragments of conjugating pairs was regular; I have never seen a large and a small cell formed by division in the original plane as so frequently happens when cells



10 11 12

FIG. 10. Conjugating cells showing region of the cut.

FIG. 11. The right and left halves of a conjugating pair after removal of the anterior end.

FIG. 12. Left and right halves of a conjugating pair grafted together.

are cut in the vegetative condition. This regularity would indicate that the division plane is lost during conjugation, and that when it is re-formed it is laid down in the center of the fragment.

In three of the operations the halves were pressed together so firmly by the edge of the knife that the fragments grew together at the cut surfaces. These grafted fragments (Fig. 12) showed great activity for a few days, but finally died without dividing. One of these was stained to see the nuclear condition. There was a large single nucleus in each half. If these grafts could be kept and fission induced, the regulation would, no doubt, be of value in throwing light on the problem of the "nucleus-protoplasm-relation."

F. *The Effect of Cutting Cells Shortly after Fission.*

In order to obtain cells at a definite period in their development they were removed from the stock culture, and as soon as they had separated each cell was isolated in a drop of the cuture fluid. A period of two to five hours was allowed for growth, then the cells were cut and the further development of the nucleated fragments observed. The power of regeneration is present in these cells, but very few recover from the operation. In the actively growing cell the cytoplasm is in a less viscid state than it is in the vegetative cells, and it is for this reason that injury to the ectosarc results, in ninety per cent. of the operations, in the escape of the entire contents of the cell. In successful operations removal of the anterior or the posterior end give the same general results as in the vegetative cells. The most striking fact brought out is that as early as two and a half hours after fission the next division plane is determined. A brief description of a few experiments will demonstrate this.

R. 28. Two sister cells were isolated two and a half hours

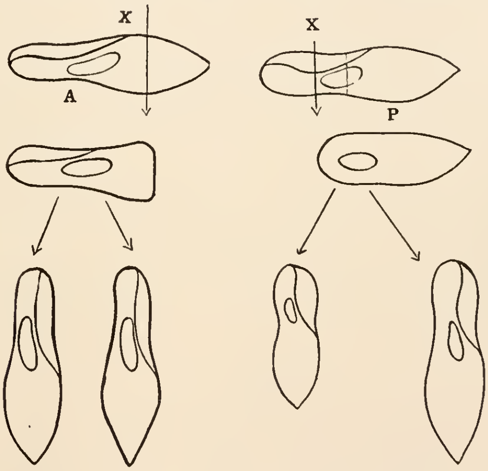


Fig. 13. Two sister cells cut at the same time, showing the formation of a normal race from the one from which the posterior end was removed, and the irregular cells formed from the cell from which the anterior end was removed.

after fission; the anterior end of one and the posterior end of the other were removed (Fig. 13, *A* and *P*). On the following day the nucleated fragments were active but had not yet divided. By noon of the same day *A* had formed two normal cells and *P* had divided into a small and a large cell. Later the smaller cells grew to the normal size; they were then killed.

R. 59. The anterior end of a cell was cut off four hours after fission. The nucleated fragment divided irregularly into a small anterior and a large posterior cell.

R. 30. One monster was obtained from a cell cut five hours after fission. Almost the entire anterior half was removed (Fig. 14, *X*). The wound closed in very slowly, the fragment increased in size (*C*), and not until the fourth day after the operation was any sign of division observed; then a constriction appeared near the anterior end which finally assumed the shape of an individual (Fig. 14, *D*). On the tenth day the mass divided into two irregular cells (*E* and *F*) each containing only one nucleus. It is evident that fission here was not only delayed but the whole mechanism of cell division was thrown out of order. The disturbance here was greater than when the cut was made further forward, and may have been due to injury to the nucleus.

In these young cells there was no sign of an increased rate of fission after removal of a part of the cytoplasm such as was observed in vegetative cells where three or four divisions followed in quick succession in the first twenty-four hours after the operation.

G. *A Comparison of the Behavior of the Nucleated Fragments from Four Different Races.*

In order to compare the power of regeneration in different races four distinct lines were selected and the following table compiled.

TABLE III.

Race.	Food.	Regeneration Followed by Normal Division.		Division in Original Plane.		No Regeneration.	
		Anterior End Removed. Per Cent.	Posterior End Removed. Per Cent.	Anterior End Removed. Per Cent.	Posterior End Removed. Per Cent.	Anterior End Removed. Per Cent.	Posterior End Removed. Per Cent.
C	M. Milk	67	100	33	9	0	0
M	M. Milk	23	25	59	30	18	45
E	Timothy	50	88	50	12	0	0
S	Timothy	25.5	43	49	30	25.5	27

Such a table as this demonstrates clearly that the power of regeneration varies greatly in different races. It also shows that in all four races there is greater disturbance when the anterior end is removed than there is when the posterior end is cut off. This table does not show the tremendous differences in the behavior which result from the condition of individual cells at the time of the operation. There is a definite correlation between the behavior of fragments and the periods of depression to which cultures are subject. At such times a race which normally shows great power of regeneration fails both to regenerate and to divide. The power to regenerate is not so much a characteristic of a race as it is an indication of the vitality of the individual cell. *Paramecium* taken from a pure line will regenerate in ninety cases out of a hundred if the cytoplasm is in a viscid state and the animals are well fed. When the cells are starved or in a period of depression from other causes, the rate of division is slow and the power of regeneration is greatly reduced or altogether lost.

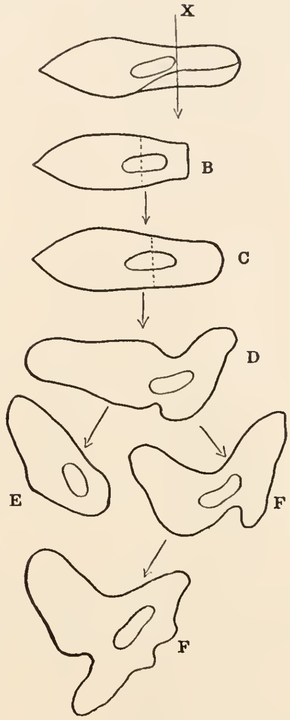


FIG. 14. Diagram giving the history of a fragment cut close to the nucleus.

H. *Can the Size of a Race be Reduced by Removal of Part of the Cytoplasm?*

In his extensive and careful investigations on *Paramecium* Jennings ('10) has shown that race size is inherited and that a new race of a different size does not arise within a pure line. Popoff ('09) claims that it is possible through experimental means to change the size of the race and to maintain the altered cell size for an indefinite period. The forms upon which Popoff made his observations were *Frontonia leucas* and *Stentor cæruleus.*

In order to determine whether or not a loss of cytoplasm alone would reduce the size of a race permanently I undertook a long series of experiments on certain pure lines where the average size was definitely ascertained before the operation was performed, and the average length and breadth of all cells arising from small fragments was compared. If these measurements had been made from cells of the first few generations arising from such fragments the conclusion would have been reached that it is not only possible, but quite a simple matter to produce a small race from a large one. It is necessary to keep such cultures for many generations until after repeated divisions the normal size is regained. The method followed in these experiments may be illustrated by giving one example. All measurements were made with a micrometer under a Zeiss objective 3, ocular 1, after the cells were killed in Worcester's fluid.

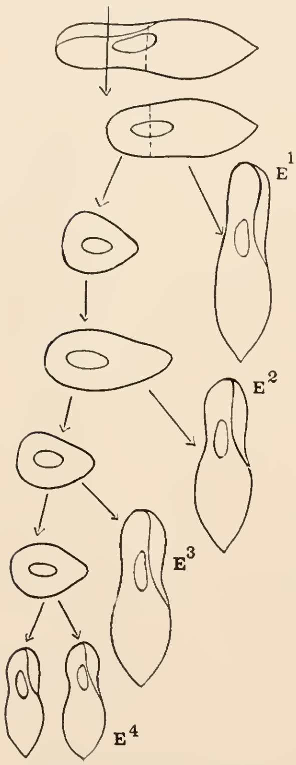


FIG. 15. Diagram showing the formation of normal races after irregular division

Experiment E.—The anterior end of the cell was removed (Fig. 15), and as soon as the nucleated fragment divided into a small and a large cell the two individuals were separated. The large piece continued to divide forming a normal race which we shall distinguish as E^1. The small piece after a period of growth divided irregularly into a small anterior and a larger posterior cell. The descendants of the posterior cell formed a race which is designated as E^2. The next division of the small piece was again irregular, the posterior cell exceeding the anterior in size. The race formed from this large cell was designated E^3. The small anterior piece at the next division formed two cells of equal size; these were called E^4. There were then four races E^1, E^2, E^3 and E^4 to be compared with the pure line E from which they arose. Careful measurements were made from time to time and during the course of the experiments, and all of the races were kept under the same conditions as the control. The experiment was begun on March 14, and the first measurements made on the twenty-third showed the same average size in E, E^1 and E^2, but E^3 and E^4 were much smaller. The next measurements made on the twenty-sixth showed that E^3 had reached the average size, and a week later the small cells of E^4 were fully as large as the control race.

It is undoubtedly true that a large number of these small fragments, and most of the cells thrown off from the monsters, show signs of extreme feebleness and often die after one or two divisions. It is only those that formed races that are taken into consideration. Small fragments if possessing enough vitality to continue the race will sooner or later become as large as the parent stock. At the present time I have a race descended from a small anterior fragment formed by irregular division. The average size of these cells is slightly larger than that of the pure line from which they arose.

In this connection it is also interesting to note that after removal of the anterior ends of conjugating cells (Fig. 10, *X*) when for some reason the fragments differ in size (Fig. 11, *L* and *R*) the descendants of both halves finally regain the same average size.

General.

In considering the phenomena of regeneration and regulation in a unicellular organism one must take into account the fact that we have, in such a form as *Paramecium*, a highly differentiated structure. This differentiation is greater in the anterior end of the cell and diminishes posteriorly. Therefore it is not surprising that less disturbance results from removal of the posterior than of the anterior end. Regeneration follows the removal of the posterior end (Fig. 1, *Z*) in most cases, but this does not mean that the entire end is replaced before fission occurs Such fragments divide before the full size is regained, therefore regeneration amounts to nothing more than the healing over of the wound and the formation of new cilia; the regaining of the original body form is brought about by a gradual process of regulation through growth in volume. After the wound is healed a new plane of division is laid down, and through fission two individuals of equal size are produced; when the new plane is not formed division takes place in the original plane and two cells of unlike size are produced. What causes this difference? Why does a fragment usually divide into two cells of equal size when the posterior end is cut off and of unequal size when the anterior end is removed? I have suggested that this is due to the position of the nucleus. When the cut is made close to the anterior end of the nucleus it is stimulated to divide at once while there is no such stimulation when the cut is made through the end of the body at some distance from the posterior end of the nucleus. Popoff claims that the volume of the cytoplasm can be regulated so that it accommodates itself to the volume of the nucleus. In this instance, however, it does not seem to be a question of balance between nuclear volume and cell volume. After removal of one end the fragment contains a whole nucleus and only two thirds to three fourths the normal volume of cytoplasm. After the first irregular division the small piece contains one half of the original nucleus and from one fourth to one third of the cytoplasm. Before the balance is established the cell may divide again into a small and a larger cell, or into two cells of the same size. When cut in half in the vegetative state the surviving fragment contains all of the nucleus and one half of the cytoplasm, yet these cell fragments divide in the normal manner.

It appears then that the ratio of the volume of the cytoplasm to the volume of the nucleus does not explain these results, and we must, therefore, look to some other source for light on this subject. If, as it appears, the division planes are laid down shortly after fission is it not possible that irregularities might result from the disturbance of these planes? I do not believe that there is any visible plane present, but there is strong indication of some such differentiation. If we consider a mature *Paramecium* as possessing a plane through the center as indicated by the double row of dots in Fig. 16 and two potential planes in the middle of each half, at the single row of dots, there are four normal individuals present in one cell. A cut made through the center should be followed by normal division of the surviving half, and this is the case. If the cut is made through either of the other planes we should expect division in the original plane resulting in a small and a large individual, but if the cut were made in front of the most anterior plane or back of the most posterior one there would be two opposing division areas in the same fragment and a reorganization would be necessary; thus both planes would disappear and a new one would form which would bring about symmetrical division. The results of the experiments that I have made seem to support this theory, but in order to test the validity of the suggestion it would be necessary to make careful measurements of each cell used, and it would scarcely be possible to determine with exactness whether or not the cut were made through the region of a division plane or on one side of it.

Fig. 16. Diagram of mature cell showing regions of division planes.

The fact that a loss of cytoplasm from such a highly differentiated organism as *Paramecium* does not affect the size of the race is one more proof of the necessity of seeking some way of reaching the nucleus, for if some of the nuclear material could be removed the result might be very different. So far I have found it impossible to keep a fragment alive after injury to the nucleus. It is possible that after repeated removal of cytoplasm from the same cell a small race size could be established, but the difficulties of the operation and the great disturbance produced by it in the cell would render the experiment practically impossible.

Summary.

1. When the anterior end of the cell was removed by a transverse cut through the peristome just in front of the macronucleus, thirty-four per cent. of the fragments regenerated the lost end and divided normally. When the posterior end was removed by a similar cut, back of the mouth, sixty-two per cent. of the nucleated fragments regenerated the lost end and formed a normal race.

2. Removal of the anterior end resulted in irregular division

in fifty per cent. of the nucleated fragments, while removal of the posterior end caused irregular division in only twenty per cent. of the pieces.

3. *Paramecia* cut in half transversely usually die as the nucleus is forced out by the pressure of the knife. When the nucleus remains in one of the halves regeneration is followed in that half by normal division.

4. Monsters develop from fragments composed of more than half of the protoplasm of a normal *Paramecium*, but never develop when a cell is cut exactly in half.

5. A fixed division plane is present in the normal cell as early as two and a half hours after fission. It is possible in vegetative cells, where fission has been delayed for a period, that more than one plane is laid down. This would account for the fact that after cutting off one end of the cell several divisions follow in quick succession.

6. The power of regeneration varies in different races, and in different individuals of the same race. It is the index of the condition of the cytoplasm.

7. The removal of a portion of the cytoplasm does not result in the production of smaller individuals. After several generations have been produced the normal size is regained.

Bryn Mawr, Pa.,
May 11, 1912.

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